

The University of Chicago

**REGIONAL DIFFERENCES IN
EYE-FORMING CAPACITY OF THE EARLY
CHICK BLASTODERM AS STUDIED IN
CHORIO-ALLANTOIC GRAFTS**

**A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF ZOÖLOGY

1935

By

L. FLOYD CLARKE

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REGIONAL DIFFERENCES IN EYE-FORMING CAPACITY
OF THE EARLY CHICK BLASTODERM AS STUDIED
IN CHORIO-ALLANTOIC GRAFTS

(Four figures and one plate)

L. F. CLARKE

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and University of Rochester

THE present investigation is concerned with relative eye-forming capacities of various regions of the chick blastoderm in stages from late primitive streak through head process up to and including early somite stages. It is proposed (1) to determine the position, extent, and boundaries of the area with potencies for eye production in late streak and head-process stages, (2) to analyze the relative eye-forming capacities of the various regions within this area, and (3) to consider the evidence for a changing organization with respect to eye-forming potentialities at different stages in development.

There is present in the blastoderm of chicks of late streak or of head-process stages a definite region with eye-forming capacities. It is possible to determine the level in the embryonic axis and to define with a fair degree of accuracy the boundaries of the eye-forming area under the conditions of chorio-allantoic grafting.

In normal development the optic vesicles arise by lateral out-pouchings of the walls of the forebrain. If we trace development backward to late streak and head-process stages, it is apparent that the prospective eye-forming areas are bilateral in position. In amphibians, Woerdemann (1929), Petersen (1924), and Manchot (1929) have shown by using vital staining technique that the eyes arise from bilaterally situated areas.

Two interpretations have been offered regarding the position and characteristics of the eye-forming areas. Spemann (1904, 1912) advanced the view from a study of *Triton*, *Bombinator*, and *Rana* that eye-forming areas are bilaterally situated and determined at the neurula stage, and that this determination extends even to specific

parts of the eye. Ekman (1914) and Fischel (1921) support Spemann's interpretation.

According to Stockard (1909, 1913), the eye area is first median and then by cell movements of eye-forming materials becomes bilateral in position. This view is based on experimental production of cyclopia in *Fundulus* and defect experiments on the neural plate of *Ambystoma*. Adelmann (1930) has shown for the urodeles that in the medullary plate stage median and lateral regions of the anterior end of the medullary plate have the capacity for eye production. These results favor the view of a single area with eye-forming potencies which includes lateral, as well as median, regions. The greatest capacity for eye production was demonstrated to lie in the median region.

The results of the present investigation demonstrate that in chick blastoderms of late streak and early head-process stages, prospective eye-forming potencies are present in median and lateral regions at the levels of the anterior part of the node and of the anterior end of the notochord, respectively. As in the urodeles, the capacity is greater in median than in lateral pieces.

A distinction must be made here between the prospective potencies and prospective significance of certain regions for eye production, since the areas showing eye-forming capacity may not coincide exactly with the areas which actually give rise to the eyes normally. The extent to which these coincide cannot be determined from the results of the present investigation. The experiments reported here deal with the potencies and are not directly concerned with the prospective value of the various areas.

Results obtained from experiments on late streak and head-process stages, namely, that median as well as lateral regions have eye-forming capacities, led to experiments in which an attempt was made to determine at what stage in development the median region loses this capacity. The results here are of interest in that up to the nine-somite stage narrow median strips from the brain floor still gave eye tissues under the conditions of the experiment.

This work represents a portion of a larger program undertaken by Dr. B. H. Willier and his students to map out the various organ germ areas of early chick blastoderms and to study their potentialities.

ties under the conditions of chorio-allantoic grafting. The advice and criticisms of Dr. Willier, who has supervised this investigation, have been responsible in large measure for the progress made. I should like to express my appreciation of his assistance. Also, I wish to express my appreciation of the excellent facilities provided at the University of Chicago and the University of Rochester which enabled me to carry out this investigation.

MATERIAL AND METHODS

The method employed is essentially that described by Willier (1924). A chick blastoderm of desired stage in development is removed from the yolk and placed in a Syracuse watch glass. The embryo is kept in 0.9 per cent sodium chloride solution at a temperature of 39°C. throughout the operation. In a watch glass the blastoderm is flattened out, and by means of an ocular micrometer measurement of the length and width of the pellucid area and the lengths of the primitive streak and head process are recorded. These measurements, together with structural characteristics, are utilized in ascertaining the stage of the donor embryo.

Fine glass needles are employed in making the desired isolations. By placing the needle on the blastodisk and applying slight pressure, clean, accurate cuts are made with a minimum destruction of tissue. Each implant thus isolated is transferred in a drop of saline solution by means of a Spemann micropipette to the chorio-allantoic membrane of a host embryo previously incubated for 9 days. The grafted tissue becomes incorporated in the vascularized membrane of the host. After a period of 9 days, during which the graft grows and differentiates, it is recovered for histological examination. The grafts were fixed in Bouin's solution, sectioned at 8 μ , and stained with Heidenhain's iron haematoxylin.

In connection with the study of the grafts preparations of 9-day normal embryos and a complete series of whole mounts of primitive streak, head-process, and early somite stages were examined. Some exceptionally fine slides of normal embryos, both serial sections and whole mounts, were loaned to me by Dr. B. H. Willier and Miss Mary E. Rawles, of the Zoölogy Department, University of Rochester.

DESCRIPTION OF DONORS AND ISOLATION OF IMPLANTS

The donors consisted of late streak, head-process, and early somite stages. The pellucid areas of late primitive streak stage used in the present experiments average 1.69 mm. in width, with a range from 1.26 to 2.18 mm., and average 2.67 mm. in length, with a range from 2.01 to 3.12 mm. The primitive streaks vary in length from 1.34 to 1.89 mm., with an average of 1.66 mm. The streaks in all instances are sufficiently far advanced to display definite primitive pits. The foregoing measurements are based on a total of 270 cases.

In the head-process stage the pellucid area varies in length from 2.52 to 3.57 mm., with an average of 3.02 mm.; in width the variation is from 1.47 to 2.31 mm., with an average of 1.80 mm. There is considerable variation in lengths of streak and head process, as this series includes all stages from the beginning process up to the first indication of a head fold. The range in length of head process is from 0.08 to 1.6 mm., with an average of 0.57 mm. The primitive streak ranges in length from 1.26 to 2.18 mm., with an average of 1.64 mm. Since the head process is gradually increasing and the streak correspondingly decreasing in length as a result of the backward movement of the node, the relative lengths of the two furnish a check on the developmental stage. The preceding measurements are based on a total of 372 cases.

The early somite stages used as donors comprise a continuous series of stages from presomite to twelve somites. This is the developmental period in which the morphogenesis of the neural tube is in progress. In the earliest stages (presomite to five somites) the neural folds are rolling up to form a tube. From the fifth to about the eighth somite stage a fusion of the neural folds occurs, and the walls of the diencephalon region are evaginating to form the optic vesicles. Foregut and heart are also in the process of formation during this period (Lillie, 1908, chap. v).

In late streak stages the primitive pit furnished a convenient center for describing the location of the various regions to be used as implants. The cuts are made at varying distances anterior, posterior, and lateral to the pit. A representative composite picture of the cuts made is shown in Figure 1. The distance of the cut from the pit is given in millimeters.

For purposes of analysis the letters "A," "Cb," "Ca," and "B" are assigned to the different levels of the blastoderm used in this study. It will be seen that there is considerable overlapping between the different levels. However, level A always refers to the region separated anterior to a transverse cut made in front of the pit. Posterior to this cut in each case is level Cb, which includes the pit level plus a level posterior to it. The anterior limit of the B level is at a variable distance posterior to the primitive pit. The region anterior to this level includes the pit level plus level A, and is consequently designated as level "Ca." The distance of the most posterior cut from the pit varies. An attempt was made, however, to make the areas of the levels involved in the isolation approximately equal.

Longitudinal cuts made at varying distances from the mid-line intersected the transverse cuts at right angles dividing the levels into corresponding left, median, and right pieces. The isolations are completed by making cuts around the anterior margin of the pellucid area.

Six pieces are thus isolated from each donor, as follows: left, median, and right from levels A and Cb or left, median, and right from levels Ca and B. In some instances, only the median and lateral pieces of one level are utilized.

Head-process stages are treated essentially the same as the streak stages, except that the anterior end of the notochord serves as the center about which the cuts are made. The designation of levels here is the same as in the case of streak stages (A, Ca, Cb, and B). Transverse and longitudinal cuts divide the area to be tested into left, median, and right pieces at these levels. The most posterior cut is made anterior to Hensen's node, except in short head-process stages, where it is necessary to include the node in the posterior level to maintain approximately equal areas in the levels (Fig. 2).

A third series of experiments, suggested by the results obtained from streak and head-process stages, was to isolate a very narrow strip from the median region of still older embryos to test its capacity for eye production. A piece 0.084 mm. average width (range from 0.06 to 0.1 mm.) by 0.4-0.5 mm. long was taken from the prospective brain-floor region of donors ranging from presomite to the twelve-somite stage. The notochord is always included in the piece taken. In

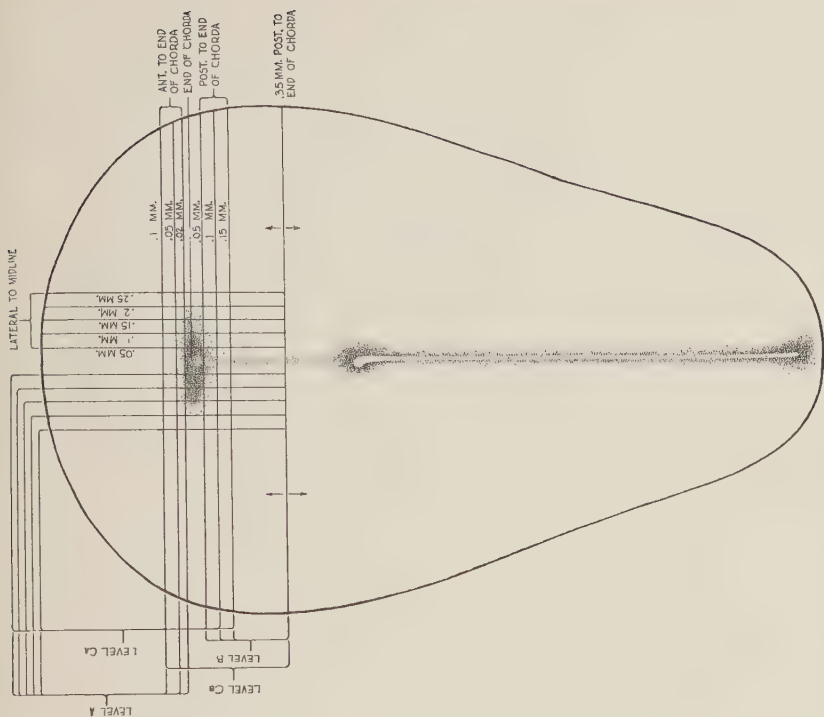


FIG. 2.—Diagrammatic representation of various cuts made in the isolation of implants at head-process stages. The position and extent of the eye area is shown. The density of stippling indicates the eye-forming frequency of various parts of the area.

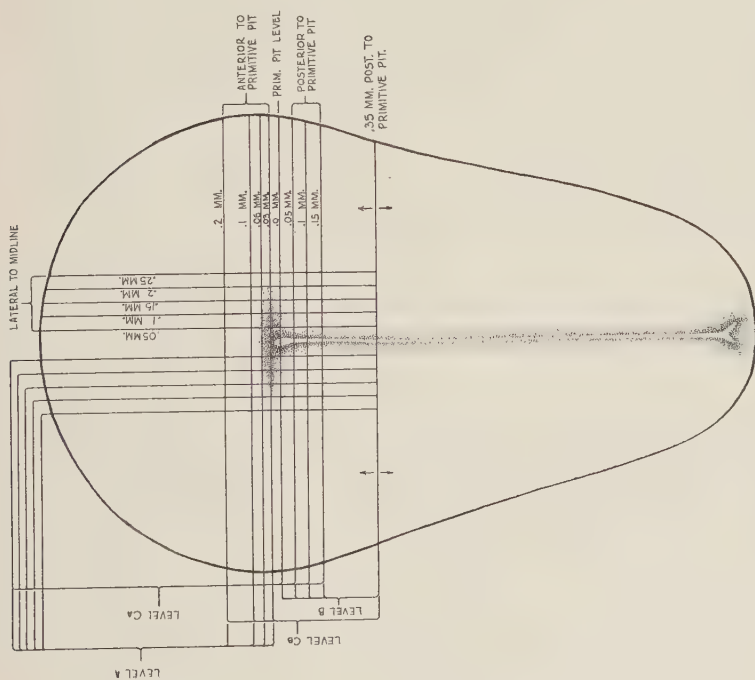


FIG. 1.—Diagrammatic representation of various cuts made in the isolation of implants at late streak stages. The position and extent of the eye area is shown. The density of stippling indicates roughly the frequency with which eye parts differentiate within the area.

some cases the head fold and developing foregut is included with the implant. After the five-somite stage the fusing neural folds must be spread apart before making the isolation.

EXPERIMENTS CONCERNING EYE-FORMING CAPACITY

I. POSITION, EXTENT, AND BOUNDARY OF THE EYE AREA

At late primitive streak stages in development.—From an examination of grafts developed from implants of various regions of late streak stages, it is determined that an area extending from the primitive pit laterally, anteriorly, and possibly posteriorly has the capacity for eye production. Figure 1 shows the eye-forming area of a late streak stage as determined by an examination of a total of 107 grafts yielding 35 cases in which eye parts differentiate.

From a total of 15 grafts obtained from implants more than 0.16 mm. lateral to the primitive pit, one shows differentiation of eye. This case resulted from a left implant of the primitive pit level isolated by a cut 0.18 mm. from the mid-line. Eight of the 13 grafts in this group were obtained from pieces 0.2–0.24 mm. from the pit. None of these 8 grafts contained eye material. It seems probable from these results that about 0.18 mm. represents the approximate lateral extent of the eye area, although the number of grafts included in the analysis is too small to establish this point.

The anterior and posterior limits are much more sharply defined than the lateral, which probably means that the level in the host axis, from which eye can differentiate, is quite definitely fixed even at this early stage in development. In 12 grafts from regions more than 0.06 mm. anterior to the primitive pit no cases of eye were recorded. Of a total of 24 grafts from 0.03 to 0.06 mm. anterior, 8, or 33 per cent, produced eye tissue. From the primitive pit to the anterior edge of the node is about 0.06 mm. Thus all these anterior regions which gave eye included a small amount of the node material.

Posterior to the pit the frequency of eye production is extremely low. Only one case has been obtained. This came from a left piece taken posterior to a cut made 0.02 mm. behind the pit level. Hunt (1932) reports no cases of eye in 25 grafts from implants taken posterior to a transverse cut through the pit.

Summarizing it may be observed that at the late primitive streak stage, any part of an ellipsoidal area extending from the primitive pit approximately 0.06 mm. anterior, 0.18 mm. lateral, and possibly 0.02 mm. posterior has the capacity to produce eye parts.

At head-process stages in development.—Figure 2 is a diagrammatic representation of the eye area as it relates to other regions of the chick blastoderm at the head-process stage in development. The position and extent of the area figured was determined by the examination of a total of 131 grafts, of which 56 contained eye tissue.

The area which has eye-forming capacities is located at the level of the anterior end of the notochord. Although this region has been demonstrated to have such a capacity by Hunt (1931), Rudnick (1932), and Stein (1933), its exact position, extent, and boundaries have not been determined previously.

An analysis of the data shows that the center of this area lies at or near the anterior end of the notochord and extends out from this center in all directions. To ascertain the extent of this area, cuts are varied in an antero-posterior and also in a mediolateral direction as described above. Measurements are made of the distance of each cut from the anterior end of the notochord. This variation is from 0.02 to 0.1 mm. anterior, from 0.04 to 0.2 mm. posterior, and from 0.05 to 0.24 mm. lateral to the anterior end of the notochord.

In the posterior direction within 0.1 mm. from the anterior end of the notochord 5 cases of eye were obtained from a total of 20 grafts, for a percentage of 25. Of these 5 cases, 1 was from left, 3 from median, and 1 from right regions. Beyond 0.1 mm. no eye tissue was present in 11 grafts.

The anterior limit is apparently closer than this to the anterior end of the notochord. From 13 grafts within 0.05 mm. anterior to the notochord 7 cases of eye were obtained. Here also left, median, and right regions are represented. Beyond 0.05 mm., however, no eye tissue was obtained from 5 grafts. Incidentally, it should be noted that graft frequency is low at this extreme anterior level.¹

In a mediolateral direction the extent of the area is not so well defined; but of a total of 11 grafts from lateral pieces, 0.16 mm. or

¹ I am indebted to Dr. B. H. Willier and Miss Mary E. Rawles for 6 grafts included in the tabulations of level A.

more from the mid-line, only 1 case of eye was obtained. This came from a left piece beyond 0.19 mm. lateral to the mid-line. Six of the grafts were from pieces more than 0.2 mm. from the middle. This case of eye was obtained from the C level (level of the anterior end of the notochord), which may mean that the lateral extent of the area is greatest at this level.

Summarizing, it is observed that eye is obtained from regions as far as 0.05 mm. anterior and 0.1 mm. posterior to the anterior end of the notochord and as far lateral as 0.19 mm. from the mid-line. From these data a map of the area with eye-forming capacity has been made (Fig. 2). The area is somewhat ellipsoidal in outline; its transverse diameter is about 0.38 mm., and its longitudinal diameter is 0.15 mm.

The number of grafts examined is too small to permit the statement that this is the absolute limit of the area with the capacity to produce eye. Such a statement could be based only on negative results in large numbers of grafts from regions outside the area outlined. However, it seems reasonable to conclude that the area defined above includes by far the larger portion of the region with eye-forming potencies, as expressed in chorio-allantoic grafts.

The limitations of the method make it impossible to determine whether a narrow strip of cells in the median line lacks the capacity to produce eye. However, grafts obtained with both pigmented and sensory layers of the retina from median strips less than 0.08 mm. wide or one-twentieth the width of the pellucid area makes it seem highly improbable that a strip lacking this capacity is present.

A comparison of the areas with eye-forming capacity in late streak and head-process stages shows that they are similar as to size, shape, and extent. However, the area is located at the primitive pit in the late streak stage and at the anterior end of the notochord in the head-process stage. When we consider the manner of formation of the notochord (i. e., by the backward movement of the primitive knot, leaving a strand of cells behind), it becomes apparent that the two positions are entirely comparable as to level in the axis. The medio-lateral extent of the area is practically the same for both stages. However, the antero-posterior extent is much greater in the

head process than in the late streak stage. This may be accounted for by the fact that during the formation of the head process there is an elongation of the blastodisk in an antero-posterior direction. The evidence presented above would seem to show then that the two areas are comparable as to position, extent, and boundaries.

II. FREQUENCY DIFFERENCES IN EYE-FORMING CAPACITY WITHIN THE EYE AREA

The eye differentiates in grafts from both median and lateral regions at the level of the anterior end of the notochord at head-process stages and the level of the node in late streak stages. The median regions have a greater capacity for eye production than do the lateral pieces. This is revealed in these grafts in two ways: first by a study of the relative eye frequencies, and second by an analysis of the morphogenesis and histogenesis of the eye.

At late primitive streak stage.—Table I summarizes the data on the relative frequencies of the various regions within the eye-forming area. From median pieces of levels A, C, and B (Fig. 1) 48 per cent, or 19 of the 40 grafts studied, contained eye material. Left pieces from the same levels produced eye material in 27 per cent, or 10 of the 37 grafts recovered; while 6 grafts (20 per cent) of the 30 obtained from comparable right regions showed differentiation of eye tissue. From the C level alone the eye-frequency is higher in both median and laterals, i. e., median 81 per cent, left 44 per cent, and right 43 per cent. However, the relation between medians and laterals remains about the same as for all levels taken together.

From this analysis, two important differences in eye-forming capacity are observed: first, the median is higher than either of the laterals; and second, the left is higher than the right. Not only is there a difference in eye-forming frequency, but a corresponding difference in frequency of grafted piece to survive. The median region is highest (45 per cent), left next (40 per cent), and right lowest (34 per cent).

Carrying the analysis still farther, it may be seen that ability of grafts to survive, as well as frequency of eye-differentiation from lateral pieces, tends to decrease as the distance of the lateral piece from the mid-line increases (Table I).

A comparison of frequency of eye differentiation in level C (the level of the primitive pit) and levels anterior and posterior to it shows a decrease in eye-forming capacity in both directions. The decline is much more rapid per given distance, anteriorly and posteriorly from the pit, than has been observed in a medio-lateral direction. This is shown in Table I, where it may be noted that from 0.03 to 0.06 mm. anterior to the pit the frequency of eye formation is 33 per cent, as compared with 57 per cent for the pit level. Posterior to the

TABLE I
EYE-FREQUENCIES FROM MEDIAN AND LATERAL PIECES AT VARIOUS TRANSVERSE LEVELS (LATE PRIMITIVE STREAK STAGE)

DISTANCE OF CUT ANTERIOR AND POSTERIOR TO PRIMITIVE PIT		LEVEL A ANTERIOR TO PRIMITIVE PIT						LEVEL C PRIMITIVE PIT			LEVEL B POSTERIOR TO PRIMITIVE PIT						TOTALS		PERCENT		AVERAGE PERCENT		
		.07-.2 MM.			.03-.06 MM.			.00 MM.			.00-.05 MM.			.06-.16 MM.									
	DISTANCE OF CUT FROM MIDLINE	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	GRAFTS	EYES	GRAFTS PERCENT	EYES PERCENT
LEFT	.16-.24 MM	3	2	0	2	1	0	13	5	1	2	0	0	9	1	0	29	9	1	31	11	40	27
	.11-.15 MM	6	2	0	11	4	2	13	8	3	7	4	0	4	2	0	41	20	5	49	25		
	.05-.10 MM	4	0	0	0	0	0	9	3	3	5	3	1	5	2	0	23	8	4	35	50		
MEDIAN	.16-.24 MM	2	1	0	2	1	1	6	3	2	0	0	0	4	1	0	14	6	3	43	50		
	.11-.15 MM	7	3	0	17	8	4	15	9	7	7	2	0	2	1	0	48	23	11	48	48	45	48
	.05-.10 MM	4	2	0	3	3	1	10	4	4	3	1	0	6	1	0	26	11	5	42	45		
RIGHT	.05-.10 MM	3	1	0	0	0	0	12	6	3	4	1	0	3	2	0	22	10	3	45	30		
	.11-.16 MM	6	1	0	15	6	0	12	5	3	6	0	0	4	2	0	43	14	3	33	21	34	20
	.16-.24 MM	2	0	0	1	1	0	10	3	0	2	0	0	9	2	0	24	6	0	25	00		
TOTALS		37	12	0	51	24	8	100	46	26	36	11	1	46	14	0	270	107	35				
PERCENT GRAFTS		32		47		46		31		30		4											
PERCENT EYES		00		33		57		9		00		33											

pit the drop is even more abrupt; i.e., to 9 per cent within 0.05 mm. of the pit. In fact, no eye material was obtained from grafts beyond 0.02 mm. posterior to it.

At head-process stage.—As described above, cuts made on each side of the mid-line at distances varying from 0.05 to 0.24 mm. isolated left, median, and right regions. By varying the cuts in this manner, it is possible to see what effect the inclusion or exclusion of a certain region has on the presence of certain structures and also on the differentiating capacities of the various areas. This method has been utilized here to test the difference in eye-forming capacity of various regions.

A tabulation of the case numbers from left, median, and right regions of levels A, C, and B according to the distance of the cuts from the anterior end of the notochord is contained in Table II. The frequencies are given in percentages. An analysis of these results shows that eye frequency is highest in the median (52 per cent), next in left (43 per cent), and lowest in right (27 per cent). There is a gradual increase in eye frequency of lateral pieces as the region includes more of the median area. On the left this increase is from 20 to 48

TABLE II

EYE-FREQUENCIES FROM MEDIAN AND LATERAL PIECES AT VARIOUS TRANSVERSE LEVELS (HEAD PROCESS STAGE)

DISTANCE OF CUT FROM ANTERIOR END OF NOTOCHORD		LEVEL A ANTERIOR TO NOTOCHORD						LEVEL C ANTERIOR END OF NOTOCHORD			LEVEL B POSTERIOR TO ANTERIOR END OF NOTOCHORD						TOTALS		PERCENT		AVERAGE PERCENT		
		.06-1mm.		.02-.05mm.		.00mm.		.04-1mm.		.1-2mm.													
LEFT	DISTANCE OF CUT FROM MIDLINE	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	HOSTS ALIVE	NUMBER OF GRAFTS	NUMBER OF EYES	GRAFTS	EYES		
LEFT	.16-24mm.	5	1	0	7	0	0	15	3	1	1	1	0	1	0	0	29	5	1	17	20	33	43
	.11-15mm.	11	1	0	5	1	0	38	11	7	1	1	0	5	2	0	60	16	7	27	44		
	.05-10mm.	0	0	0	3	1	1	26	15	9	8	4	1	7	3	0	44	23	11	52	48		
MEDIAN	.16-24mm.	4	1	0	5	2	2	17	10	7	2	1	0	0	0	0	28	14	9	50	64	42	52
	.11-15mm.	9	1	0	6	1	1	32	14	10	2	1	0	5	1	0	54	18	11	33	61		
	.05-10mm.	0	0	0	3	2	1	23	11	4	12	6	3	8	3	0	46	22	8	48	36		
RIGHT	.05-10mm.	0	0	0	5	3	2	20	5	3	6	3	0	6	1	0	37	12	5	32	42	30	27
	.11-15mm.	9	1	0	6	1	0	29	10	3	2	2	1	3	1	0	49	15	4	31	27		
	.16-24mm.	6	0	0	4	2	0	14	3	0	1	1	0	0	0	0	25	6	0	24	00		
TOTALS		44	5	0	44	13	7	214	82	44	35	20	5	35	11	0	372	131	56				
PERCENT GRAFTS		11		30		38		57		31		36											
PERCENT EYES		00		54		54		25		00		43											

per cent, and on the right from 0.0 to 42 per cent. As the size of the lateral piece increases, the corresponding median decreases. This results in a corresponding decrease in frequency in the median pieces. The difference in frequency may be due to the small size of the narrowest median piece grafted.

Parallel with the differences in eye frequencies are differences in graft frequencies, particularly between median and laterals; i.e., median pieces produce grafts in 42 per cent of cases, left in 33, and right in 30. That this is not merely a chance occurrence is emphasized by corresponding differences observed in similar transplants

of late streak stages. Apparently, medio-lateral differences exist in the capacity to produce graft, as well as in capacity to differentiate eye parts.

An analysis of the differences in eye-forming capacity of different levels emphasizes the importance of level in determining potentialities for organ formation. A comparison of eye-forming frequency of grafts from the level of the anterior and of the notochord (C level) and levels anterior (A level) and posterior (B level) to it shows that there is a marked decrease in both directions from the level of the anterior end of the notochord. Eighty-two grafts from level C gave eye material in 44 cases (54 per cent), as compared with 18 grafts yielding 7 cases of eye from level A and 20 grafts from level B, 5 of which contain eye. These figures include grafts from left, median, and right pieces isolated by cuts less than 0.1 mm. either anterior or posterior to the anterior end of the notochord.

These results, when combined with those described above for medio-lateral differences, show a grading-off in all directions from a region of relatively high eye-forming capacity to regions which entirely lack this capacity (Table II). The narrow antero-posterior extent of the eye area, plus the fact that the limits in both directions are quite well defined, indicates that the level for eye production has been established before this stage in development.

Figure 3 is a diagrammatic representation of these differences in terms of the distance from the mid-line of the cuts which separated the respective left, median, and right regions. The data used in constructing these graphs are summarized in Tables I and II. Although the curves do not show a smooth grading-off from the mid-line laterally, the tendency in each case is clearly indicated. The drop in frequency of formation of eye tissue from right regions is much more abrupt than from left regions, although a definite decrease is evident in both directions. The percentage values for corresponding regions in the two stages do not agree in all instances; but a definite similarity in the general trend of the two curves is evident; i.e., highest in the median, intermediate in height on the left, and lowest on the right.

Curves of the relative frequencies of the different levels from streak, as well as head-process, stages are shown in Figure 4. The

sharp decrease in each case is probably indicative of the importance of level in the host axis in determining the structures which differentiate. The difference in the sharpness of the decline in the two

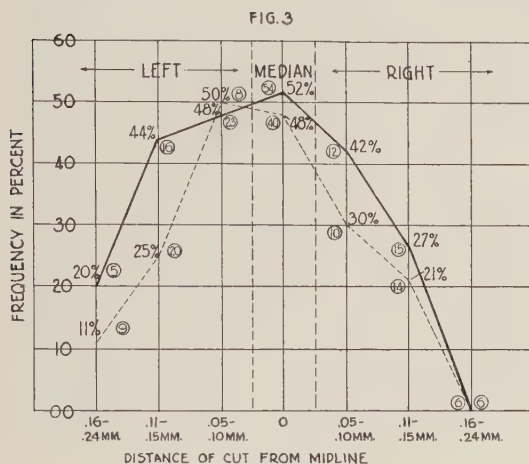


FIG. 3.—Graphic representation of the medio-lateral gradient in eye-forming frequency at late streak and head-process stages.

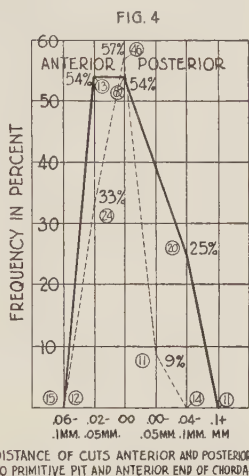


FIG. 4.—Graph showing the relative eye-forming frequencies of levels anterior and posterior to the primitive pit in late streak stages and the anterior end of chorda in head-process stages. (Late streak stages are represented by broken lines, and head-process stages by unbroken lines. The encircled figure is the number of grafts examined.)

cases may be accounted for partially at least by the fact that the blastoderm elongates considerably in the head-process stage, whereas the width is not increased proportionately.

III. EYE-FORMING CAPACITY IN EARLY SOMITE STAGES

The percentage of eye frequency for median regions at late streak stages (Table I) is 48, as compared with 52 at head-process stages (Table II), an increase of 4 per cent; while for left regions an increase of 17 per cent (from 27 to 43) and for right regions an increase of 7 per cent (from 20 to 27) is observed. A graphic representation of this relative difference between medians and laterals at these two developmental periods is contained in Figure 3. This figure is evidence of a gradual increase in eye-forming capacity in lateral regions. Both median and lateral regions show greater eye-forming capacity

in the later stages, but the rate of change is more rapid in the laterals than in the medians.

This line of evidence led to an analysis of the eye-forming capacity in younger and older stages than those just analyzed. The question as to how eye-forming potentialities of median and laterals compare in stages younger than those described above remains for future investigation. An attempt has been made, however, in the present investigation to analyze the condition in embryos still older than those considered above. For this purpose donors varying in age from presomite² to twelve somites were used. A piece of the brain floor was taken out at the level of the forebrain, and usually extending poste-

TABLE III
EYE-FREQUENCIES FROM BRAIN FLOOR GRAFTS

STAGE	HOSTS ALIVE	No. OF GRAFTS	No. OF EYES	PERCENT GRAFTS	PERCENT EYES
1-3 SOMITE	17	6	4	35	67
4-8 SOMITE	17	7	5	41	71
9-12 SOMITE	9	4	0	44	00
TOTALS & AVERAGES	43	17	9	40	53

IMPLANTS RANGED IN WIDTHS FROM .063-.10MM.

rior to it. Pieces grafted were from 0.06 to 0.10 mm. in width by 0.4-0.5 mm. in length. They include in each case the underlying notochord. In some cases the head fold and foregut material was removed, and in others it was transplanted with the brain-floor material. What influence, if any, the removal of foregut has on the differentiation of eye is not revealed by the present experiments. Eye differentiated in grafts from pieces without foregut material.

It is of interest to note that all of the grafts resulting from this extremely small implant are very small, but the differentiation is not noticeably different histologically from that in much larger grafts.

For convenience in making comparisons the grafts are divided into three groups according to the stage of the donor from which they came. Table III summarizes concisely the results obtained from each of these groups. The data show in the first place that eye is produced with a relatively high frequency in stages from presomite to eight somites. The percentages are approximately the same for the presomite to three-somite and the four-to-eight-somite groups. Secondly, it will be observed that from nine-to-twelve-somite stages

² "Presomite" is used here to refer to the stage in which the neural folds are beginning to arise but before the first somite is definitely indicated.

no cases of eye were obtained from 4 grafts. This result indicates that the eye-forming capacity, which is present in the brain-floor region from presomite to eight-somite stages, is lost during the following stages. A great many more grafts would be necessary to establish this point, but the results are of sufficient interest to justify their inclusion in this paper. It has been demonstrated, at least, that the capacity for eye production remains in this median region, even up to the stage at which the optic vesicles are beginning to form. A definite isolation of the potencies into the lateral primordia does not precede the beginning of the morphogenesis of the optic vesicles.

It is interesting to note here that in a series of 6 grafts of the lateral region, which is prospective eye material, eye tissue was present in every graft. When this result is compared with that for head-process stages, it becomes evident that a definite rise in the eye-forming potentiality of the lateral regions has occurred during this developmental period.

IV. QUALITATIVE DIFFERENCES WITHIN THE EYE AREA AS SHOWN BY A STUDY OF MORPHOGENESIS AND HISTOGENESIS

Regional differences in capacity to produce eye is very evident in the grafts studied in the present investigation. It is not my purpose in the present paper to consider in detail the qualitative differences in the various regions, but to describe more or less typical cases and to summarize the differences in capacity for differentiation of the regions under consideration.

Eye tissues differentiating in grafts do not display normal relationships, probably because of mechanical factors operating during morphogenesis. The histology, however, is essentially like that of normal tissues. This observation is substantiated by comparing the results of Weyssse and Burgess (1906) on the normal histogenesis of the retina and of Hoadley (1925) on the histology of eye tissues in grafts.

Analysis of regional differences in eye-differentiating capacity in grafts from both head-process and late streak stages shows that the median region has a much greater capacity to form essential eye tissues consisting of sensory and pigmented layers of the retina and lens than do the lateral regions. However, in grafts from median regions all degrees of differentiation are present from a small amount of

PLATE I

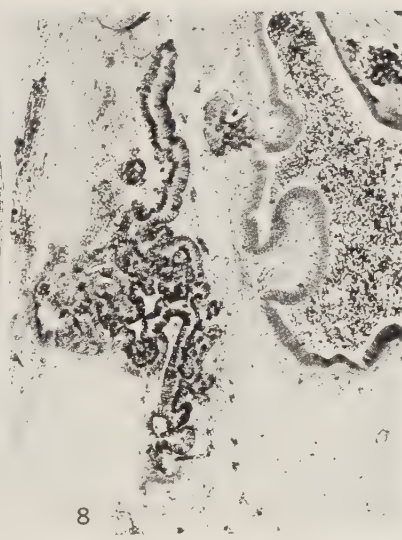
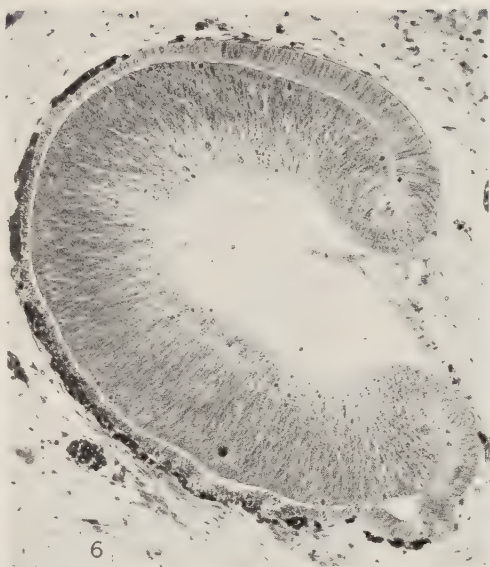
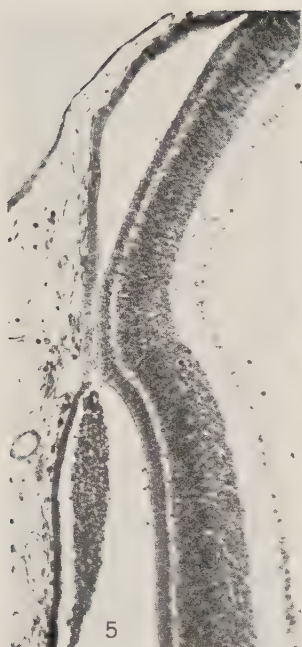
FIG. 5.—A portion of an optic cup showing the various layers of the differentiated retina. The implant was a median piece of donor at head-process stage (see text for details). $\times 235$.

FIG. 6.—An optic cup showing both pigmented and sensory layers of the retina; differentiated from a median piece of donor at late streak stage (see text for details). $\times 210$.

FIG. 7.—A large optic vesicle showing lens in contact with convoluted pigmented layer; differentiated from a left anterior piece of donor at head-process stage (see text for details). $\times 38$.

FIG. 8.—A portion of a graft showing convoluted pigmented layer connected with forebrain material; differentiated from a right anterior piece of donor at head-process stage (see text for details). $\times 140$.

PLATE I



pigmented layer to a definite optic cup with the differentiated retina. In Figure 5, a section of such an eye cup is shown. The donor from which the grafted piece was taken was in the head-process stage. The implant included the anterior 0.1 mm. of the notochord, 0.1 mm. on each side of the mid-line and extending anteriorly to the margin of the pellucid area.

Out of a total of 28 cases of eye tissue recorded from median pieces, 6 showed a differentiation comparable to the above mentioned case. In several other cases a much thickened layer, presumably retina, was found connected with the pigmented layer.

Figure 6 is a photomicrograph of a relatively normal-appearing optic cup. The donor in this case was at a late streak stage. The piece used as the implant was taken 0.06 mm. anterior to the primitive pit and included 0.10 mm. on one side and 0.12 mm. on the other side of the mid-line, as determined by the position of the pit. The piece extended anteriorly as far as the margin of the pellucid area.

Differentiation has not progressed as far as in the preceding case described, but the sensory layer is cupped back into a pigmented layer. In no instance was differentiated sensory layer obtained from late streak stages. This is probably a chance occurrence, as complete differentiation has been obtained from stages much younger than these (Butler, 1935).

The best cases of lens differentiation have been obtained from median regions. Three such cases have been positively identified. It is interesting to note that the ectoderm included with the implants in these cases does not normally contribute to the lens. In each of the three cases the lens is connected to skin in the graft. One possible explanation of the occurrence of lens in these grafts would be induction by the eye differentiated in the graft. In this connection it is also interesting to note that the eye tissue is derived from cells which normally do not contribute to eye formation.

When we proceed now to a consideration of the type of differentiation obtained from lateral pieces, it may be observed that no case of differentiated sensory layer has been recorded from any lateral region. However, definite vesicles are common from left lateral regions. Figure 7 shows a large vesicle entirely surrounded by pigmented layer, with a small lens shown at one point in contact with

the pigmented layer. This lens is continuous with definite skin in another section. Also in another section the pigmented layer is continuous with a cupped sensory tissue which is connected with the forebrain material seen in the center of the vesicle.

Figure 8 is quite typical of the differentiation found in right regions. Pigmented layer is present in a much convoluted form with no definite organization into an eye. It seems significant that in no case was a vesicle of the type quite common to grafts from left and median regions found.

It is also interesting to note that the quantity of eye material is smaller from right pieces than from other regions tested, and that in no case has lens been identified in grafts from right pieces but that three cases have been noted from left. The eye material is shown connected to a forebrain vesicle in the figure. This connection is typical in grafts obtained from left and median, as well as right, regions. The presence of eye in connection with nervous tissue is used as a criterion for identifying forebrain (Willier and Rawles, 1931).

By the foregoing analysis of the differentiation of eye tissue in grafts from median, left, and right regions, additional evidence is furnished for some fundamental difference in these three regions. The median region produced the highest frequency of both eye tissue and grafts and the best differentiation of eye in the graft. Furthermore, the percentage of graft survival, as well as eye formation and the type of differentiation obtained, was higher from left than from corresponding right regions. The fact that all three lines of evidence point in the same direction indicates that we are dealing with a general difference in activity of the three regions. This may be expressed as a medio-lateral gradient with a more rapid decline to the right than to the left.

DISCUSSION

The analysis of results has brought forth three generalizations with respect to eye-forming capacities of early chick blastoderms. First, in late streak and head-process stages an area is present more or less definitely localized and limited, any part of which has a capacity to produce eye tissue. Second, within this area there exists a medio-lateral, as well as an asymmetrical, difference in eye-forming capacity. Third, during the normal developmental processes there is

a progressive increase in the eye-forming capacities of lateral regions (the regions which normally produce eye). The median region (prospective brain-floor material) does not lose this capacity until after the eight-somite stage.

I. THE NATURE OF THE AREA WITH EYE-FORMING CAPACITY

It has been determined that the entire blastoderm at head-process stages in development is composed of organ-forming germ areas Rawles and Willier, 1934; (Willier and Rawles, 1935; and Rawles, 1935). The potential eye area then is only one of a great many such areas which overlap each other.

It has been shown previously that eye-forming capacity is present in different regions of the blastoderm at late streak stages (Hunt, 1932; Stein, 1933) and at head-process stages (Hunt, 1931; Rudnick, 1932; Stein, 1933). The present investigation has attempted to localize more definitely and to limit these regions.

The eye-forming germ area occupies a somewhat comparable position in both late streak and head-process stages. At late streak stages the primitive pit and at head-process stages the anterior end of the notochord are centers about which materials have eye-forming capacity. In both, the region with eye-forming capacity is ellipsoidal in outline with the greater diameter at right angles to the longitudinal axis of the embryo. The longitudinal diameter at late streak stages is much shorter than it is at head-process stages. Cells within these ellipsoidal areas have eye-forming capacities. In addition, these same cells may have the potentiality for various other ectodermal structures characteristic of the given level or region in which they are located. The determination process up to this time, then, is probably concerned only with restricting the area with potentialities for eye production, and not specifically and irrevocably determining certain cells for a specific organ. Potential forebrain material occupies approximately this same region and is found associated with eye in grafts from implants of any part of this area. Apparently, any part of the region, then, has the capacity to produce more than it does produce normally.

Since both sensory and pigmented layers of the retina and lens can be produced from various parts of this region, it is likely that the area is an equipotential system for eye production; i. e., any part is

capable of producing a complete organ, though very much reduced in size. Spemann and Bautzmann (1927) and Holtfreter (1931) point out that the presumptive medullary plate is equipotential. Adelmann (1929*a*, 1929*b*, 1930) has shown that in the urodeles the anterior end of the medullary plate is an equipotential system. In his experiments median or lateral regions of the anterior medullary plate were removed, and the remaining parts developed eyes. Also, these same areas transplanted separately to the belly wall produced eyes. By studying the eye-forming frequency of median and lateral pieces, Adelmann found that median regions have a much greater capacity for eye production than do laterals.

On the basis of what has been said, we cannot consider the eye area in chick embryos of these stages as either localized distinctly median (Stockard, 1913) or as bilateral primordia specifically determined (Spemann, 1912), but as an area occupying both median and lateral regions of the prospective neural plate material at the level of the primitive pit or the anterior end of the head process. The prospective eye regions in the chick are probably bilateral, as Woerdemann (1929), Petersen (1924), and Manchot (1929) have demonstrated for amphibians; but this certainly does not refer to potential singleness or doubleness of the eye anlage.

II. MEDIO-LATERAL AND ASYMMETRICAL DIFFERENCES IN EYE-FORMING CAPACITY

It has been pointed out that the median regions produce both grafts and eyes with greater frequency than do the laterals. Also, the type of differentiation of eye in grafts from median regions is better than from the laterals, as based on the quantity of eye material, the degree to which differentiation has progressed, and the relative normality of the eyes. It has been noted that a corresponding difference is present between left and right regions; the left is superior to the right in graft and eye production, as well as in type of differentiation.

The fact that the variation is in the same direction; i. e., best in median, next in left, and poorest in right, with respect to all three of these conditions in both late streak and head-process stages furnished additional evidence that some one generalized condition is responsible for all three results.

Graft frequency can be related to a difference in the capacity of the implants to become incorporated in the chorio-allantoic membrane or to differentiate once they become incorporated. Assuming a more or less constant medium of differentiation, the difference in the capacity of the various regions to produce grafts is a function of the activity of the cells implanted. Similarly, the capacity a graft has to produce all organs for which it has potentialities or to produce a particular organ every time depends on the general ability of the cells to express all their potentialities. This would determine the frequency with which any organ would occur in grafts.

Hyman (1927) has shown by susceptibility experiments that the node region in late streak and the corresponding region of the anterior end of the head process in head-process stages are the regions of greatest metabolic activity. The results of the present experiments bear this out, using the capacity for eye production as a measure of this difference. In addition, the present experiments indicate strongly that the left side is more active than the right; but on each side there is a gradual decrease in activity in a medio-lateral direction. Using eye frequency as a measure, there is a medio-lateral gradient in cell activity, with the decline more rapid to the right than to the left.

On the basis of the discussion just presented, Figure 3, showing graphically the relative eye frequencies of left, median, and right regions, may be offered as an indication at least of the relative differences in activity of the three regions.

III. EVIDENCE OF A CHANGING ORGANIZATION

In the preceding discussion an analysis has been made of the differences which exist in different parts of the eye area at head-process and late streak stages in development. This leads next to a consideration of the differences which exist between successive stages in the developmental process.

The data presented in the preceding sections give evidence of a regular and orderly change in the eye-forming capacities as development proceeds. An increase in the eye-forming capacity of lateral regions at head-process stages over lateral regions at late streak stages is indicated by comparison of eye frequencies. Also, the in-

crease observed in median regions from late streak to the head-process stage is not nearly as great as the lateral increase (cf. Tables I and II). When head-process stages were compared with still later stages; i. e., early somite stages, it was found that the capacity of the lateral regions (potential optic vesicles) had increased to 100 per cent, whereas the corresponding medians (potential brain floor) did not increase to more than 70 per cent. In stages beyond eight somites no eye tissue was present in 4 grafts from the brain floor.

These results are interpreted as indicating a shift in developmental potency for eye production from the median to the lateral regions. This process probably continues until the median region no longer has any eye-forming capacity, while the lateral regions produce eye every time the transplanted tissue develops into a graft. This is probably the stage in which the prospective eye areas attain specific potencies (Lillie, 1929). From this stage on, eye tissue alone would be produced by these areas, according to this interpretation.

During a comparable stage in urodeles prechordal mesoderm is shifting laterally in position, and mandibular arch material is being added from the neural crest (Adelmann, 1934). Cyclopia accompanies the failure of the prechordal mesoderm to shift laterally. Probably the activity of the mesoderm material brings about an increase in the activity of the lateral regions to an optimum for eye production, and consequently the greatest eye-forming capacity becomes located in the bilateral optic vesicles.

This explanation is supported by certain experiments in producing eye abnormalities in *Planaria dorotocephala* (Child, 1916, 1920, 1921, and 1924, pp. 107-10). By subjecting the animals to inhibiting agents during regeneration, it was found that the medio-lateral gradient could be inhibited to varying degrees so that the median region with the highest metabolic rates would be reduced and the bilateral organs approximated to the median line. In other words, the median region now assumes the metabolic rate characteristic of the bilateral organs (in this case eye), so that the eyes differentiate at or near the mid-line. If this interpretation is correct, there is a shift of eye-forming potentialities toward the mid-line as a result of an inhibition of the medio-lateral gradient; whereas in normal development the potentially single eye-forming area becomes divided into

bilateral primordia as a result of a stimulating influence exerted on the ectoderm, probably by increased mesodermal activity.

Wright and Wagner (1934), in accounting for head abnormalities in guinea pigs, picture the existence of various centers of activity at certain critical stages in development. The optic field, which is single and median, is one of these centers. If inhibitions, either genetic or environmental, are supplied to this center at the critical period, the physiological processes by which this field produces bilateral eyes is interrupted, resulting in varying degrees of cyclopia, depending on the strength of the inhibitor and the exact time of its operation.

This analysis of the cause of cyclopia is interesting in the light of the present investigation, which has shown that the median region in early developmental stages of chick blastoderms has the greatest capacity for eye production. If certain physiological processes necessary for the production of bilateral eyes were inhibited, the result would be a morphological expression of the physiological condition in existence at the time of the inhibition; i. e., the production of a single median eye. Should an inhibitor be applied at a stage in which the eye-forming capacity is greatest in the lateral regions (during the process of the formation of the optic vesicles in the case of the chick), the result possibly would be a reduction in the size of the bilateral eyes, microphthalmia, and not cyclopia. Wright and Wagner (1934) have pointed out that an inhibitory process after the separation of the two optic primordia is probably the cause of microphthalmia.

SUMMARY

1. Grafts from implants from various regions of the pellucid area of embryos at late streak and head-process stages in development have been examined for eye production. In late primitive streak stages, an eye-forming area has been found which extends 0.06 mm. anterior, 0.02 mm. posterior to the primitive pit, and 0.2 mm. on each side of it. A corresponding area is located for head-process stages, extending 0.05 mm. anterior, and 0.1 mm. posterior to the anterior end of the head process, and 0.2 mm. on each side of it.

2. Both median and lateral regions within this area have the

capacity to produce the essential components of the eye cup, retina and pigmented layers, and a lens.

3. As a result of a comparative study of eye-forming frequency and type of differentiation in grafts from median and lateral regions, it seems apparent that there is a medio-lateral gradient in capacity for eye production, a greater capacity residing in left than in right regions.

4. During the developmental process a change occurs in the relative developmental capacities of median and lateral regions for eye production. As a consequence, at early somite stages the capacity of the lateral regions (primordia of optic vesicles) is greater than that of the median (primordium of the brain floor). The median region of the brain floor retains its potentiality for eye production up to the eight-somite stage, beyond which this potency is apparently lost.

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